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Blackening of *Diospyros* genus xylem in connection with boron content

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Abstract The amount of boron contained in the xylem of Japanese persimmon (*Diospyros kaki* Thunb.) and ebony (*Diospyros ebenum* Koen) was determined by inductively coupled plasma (ICP) emission spectrometry and a modified curcumin–acetic acid method. The boron content was compared between the heartwood and sapwood of ebony as well as between a blackened portion ("kurogaki") and normal portion of Japanese persimmon. The kurogaki contained a higher level of boron than the normal portion of the same individual, although the boron content varied among individuals. Moreover, the boron content of the heartwood of ebony was much higher than that of the sapwood. These results suggest the participation of boron in the blackening of Japanese persimmon. Because both kurogaki and heartwood of ebony are durable to fungal attack, the blackening of *Diospyros* genus appears to be related to the formation of defensive substances in which boron seems to take part. The convenient curcumin–acetic acid method is an alternative to the ICP method with comparable accuracy.

Key words Boron · Curcumin method · Ebony · ICP analysis · Japanese persimmon

Introduction

The xylem of Japanese persimmon (*Diospyros kaki* Thunb.) does not change color with the formation of heartwood, and thus it is difficult to distinguish the heartwood from the sapwood. However, a blackening phenomenon occurs on rare occasions. The blackened portion (or whole timber partly blackened) has been called "kurogaki" and is highly esteemed as a material for traditional industrial arts. Yasue et al.[1] analyzed the extractives of kurogaki, and found several substances related to the blackening; however, the mechanism of and reason for the blackening have not been elucidated. In a previous report,[2] the authors compared physical, mechanical, and biodegradative properties, and levels of certain trace elements between normal and blackened portions. The blackened portion was found to be durable to fungal and termite attacks. Furthermore, a trace element analysis using inductively coupled plasma-mass spectrometry (ICP-MS) revealed that the boron content was higher in the blackened portion, which suggests a contribution by boron to the blackening.

The blackening of Japanese persimmon usually accompanies decay of the portion neighboring it, and is found on the inner surface of cut or snapped branches. The authors speculate that the Japanese persimmon tree synthesizes substances in the cell wall to protect it from fungal attack when it suffers wounding and the blackening is caused by an enzyme reaction catalyzed by boron.

However, to date, a relation between the blackening of persimmon and boron content has been found in only one individual. In this study, we clarify the causality of the blackening and portions affected by using methods focused on the quantitative analysis of boron content. In addition, we analyzed an ebony (*Diospyros ebenum* Koen) sample that also belongs to the *Diospyros* genus but whose heartwood is always black independent of injury, and compared the boron content between heartwood and sapwood.

Materials and methods

Samples

Seven samples of Japanese persimmon (*Diospyros kaki* Thunb.), which contained partly blackened portions, and one sample of ebony (*Diospyros ebenum* Koen) were examined. The persimmon samples were numbered 1 to 7, and their details are as follows.

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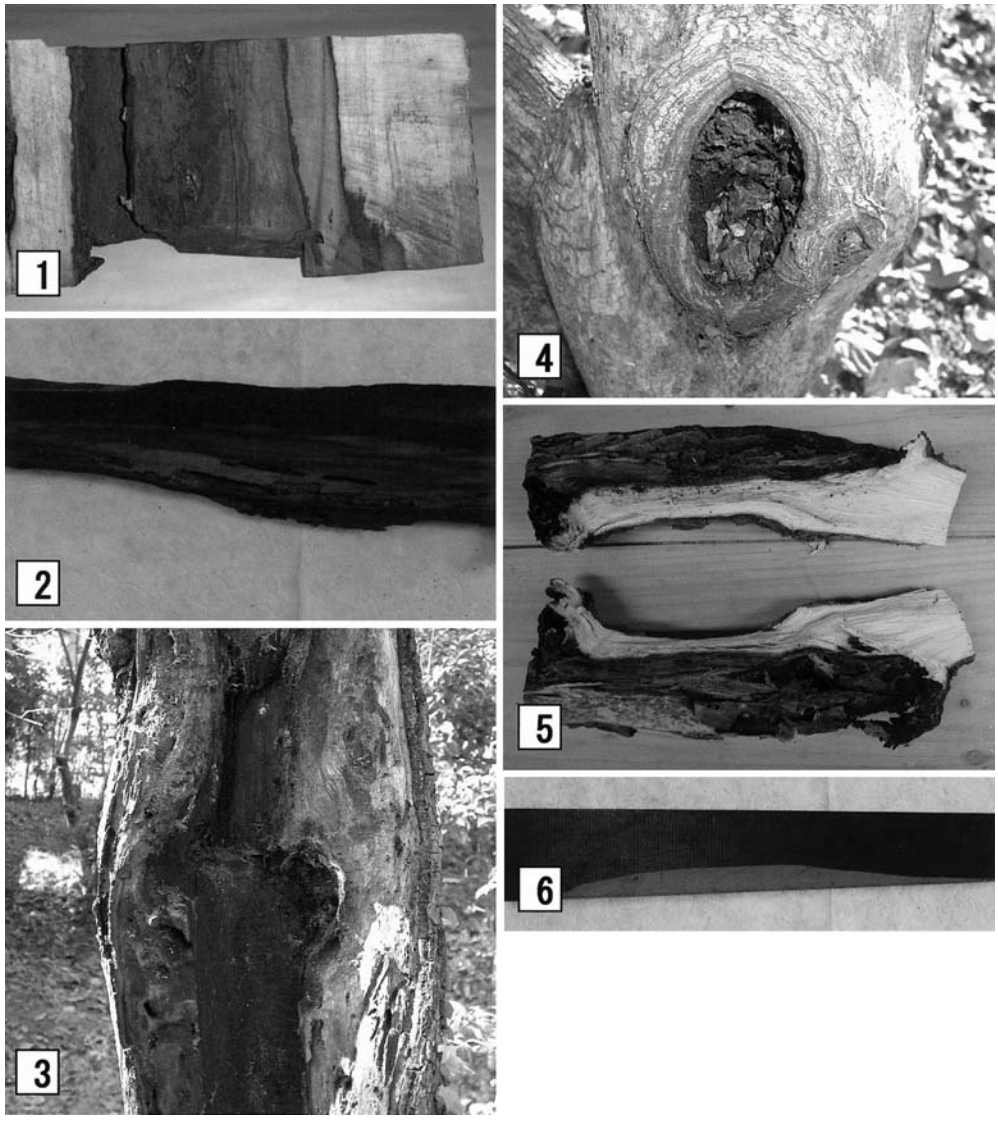

Fig. 1. Japanese persimmon samples subjected to the determination of boron content. **1** Original block of sample 1, **2** original block of sample 2, **3** original tree of samples 3 and 4, **4** original tree of samples 5 and 6, **5** original branch of sample 7, **6** ebony

Sample 1 (Fig. 1-1) was grown in the garden of a private home in Hiyoshi-cho, Kyoto Prefecture, and was cut in February 1999. The height and diameter at breast height (DBH) were about 13 m and 40 cm, respectively. Sample 1 was cut from another portion of the same log examined previously.[2]

Sample 2 (Fig. 1-2), about 40 cm long and 3 cm thick, was supplied by Miyazaki Mokuzai Kogyo, but the source is unknown. Pitch-black, dark brown, and partially decayed portions were alternately distributed parallel to the longitudinal direction.

Samples 3 and 4 came from a living tree in the garden of a private home in Tanba-cho, Kyoto Prefecture (Fig. 1-3). The estimated age and DBH are 150 years and about 40 cm, respectively. The samples were obtained from the tree at breast height. The decay proceeded from the joint of a broken branch, where the stem of the tree became hollow. The decayed portion fell away as powder and the surface of the remaining portion was blackened. Sample 3 was a thin and slender black fragment [roughly 0.5 (R) × 5 (T) × 25 cm (L)] found in the hollow, and sample 4 was whittled away from the blackened surface.

Samples 5 to 7 were obtained from the Kyoto Prefectural University Farms. Sample 5 was shaved from the hypertrophied structure developed at the periphery of a joint of a branch after pruning. The cut end became hollow and the surface was blackened (Fig. 1-4). Sample 6 came from a black lump inside the hollow. Sample 7 (Fig. 1-5) was obtained from a pruned branch whose cut end was partly blackened.

The ebony (Fig. 1-6) was supplied by Miyazaki Mokuzai Kogyo. The source is unknown.

Preparation of samples

Each sample was classified by color: whitish, brown, dark brown, and black (some were not always separated into

four groups), and broken to half the size of a matchstick with a chisel. About 500 mg of each sample was precisely weighed after being dried for 24 h at 105°C, put into a ceramic crucible, and ashed by heating in an electric furnace for 5 h at 420°C.

Determination of boron content by ICP emission spectrometry

The ashed samples were moistened with a small amount of ultrapure water, dissolved by adding 1 ml of 6 M HNO_3 (analytical grade for heavy metals, Nacalai Tesque), and then left for 30–60 min at room temperature. The solutions were transferred to 10-ml volumetric flasks through syringe filters (Cosmonice Filter W: pore size 0.45 μm, diameter 13 mm, Nacalai Tesque), and then the volumes were adjusted to 10 ml with ultrapure water.

All equipment was dipped into a detergent and then 1 M HNO_3 for 1 day, and washed with ultrapure water. All of the reagents were of analytical grade except where specified. Contact with glass vessels for long periods was avoided by using polypropylene-made vessels.

The boron content of the solution was analyzed using an ICP emission spectrometer (Seiko Instruments, SPS1500VR) at a wavelength of 249.8 nm.

Determination of boron content by a modified curcumin–acetic acid method

Sample preparation was based on the method proposed by Yamada and Hattori.[3] That is, an ashed sample prepared in the manner stated above was dissolved in 2 ml of 6 M HCl, and 1 ml of the solution was diluted to 25 ml with ultrapure water. The solution was extracted with 4 ml of 2-ethyl-1,3-hexanediol (Nacalai Tesque) and chloroform mixture (v/v = 1:4) for 3 min by shaking. The aqueous phase was removed with a syringe and then phase-separating filter paper (Whatman 1PS). To 1 ml of the organic phase, 1 ml of a curcumin–acetic acid solution, which was prepared by dissolving 0.1 g of curcumin (Nacalai Tesque) to 50 ml of hot (60°C–70°C) acetic acid, and 0.25 ml of concentrated H_2SO_4 were added, left for 30 min, and the volume was made up to 50 ml with 99% ethanol. The absorbance of the solution was measured with a spectrophotometer (Shimadzu, UVmini-1240) at 550 nm. The calibration curve was prepared by diluting the boric acid. All of the reagents were of analytical grade and the solutions were temporarily stored in polypropylene-made vessels.

Results and discussion

Boron content of Japanese persimmon and ebony

Figure 2 shows the boron content of each sample determined by ICP emission analysis. The boron levels in whitish portions were around several parts per million (ppms) except for sample 6, while those in blackened portions ranged from several to several dozen ppm. For samples 6 and 7, the boron contents in dark brown and black portions were higher than those in the other samples. These samples were obtained from different persimmon trees growing at the same place and belonged to the same breed. The boron levels recorded in the literature are 10, 2–270, and 11–140 ppm in crust, soil, and woody plants, respectively.[4] Therefore, the values obtained here may be reasonable. It is known that the uptake of boron differs depending on the kind of plant, breed, and boron content of the soil,[5] and hence the variation in boron content among individuals may be influenced by these factors.

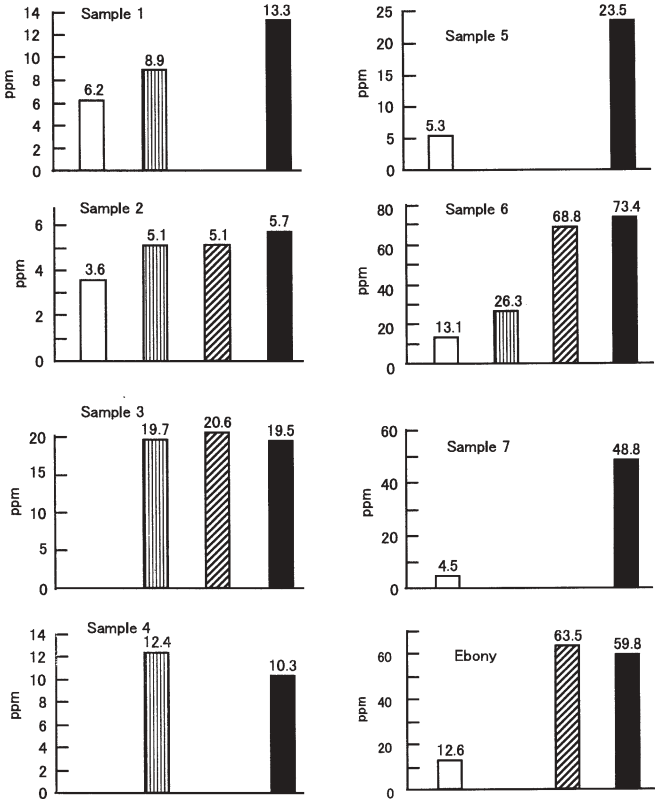

Fig. 2. Boron content of Japanese persimmon (samples 1–7) and ebony. *open bars*, whitish regions; *vertically shaded bars*, brown regions; *diagonally shaded bars*, dark brown regions; *filled bars*, black regions

The boron content was two to ten times higher in the whitish portion than in the blackened portion, although the concentration level differed among samples. For the intermediate regions, namely the brown to dark brown regions, the boron content was not always related to the darkness. This may be a result of the arbitrary nature of the classification and/or obscurity of the boundary. Nevertheless, for samples 1, 2, and 6, the boron levels were higher in the brownish portions than in the whitish portions, and were lower in the brownish portions than in the blackened portions.

The boron content of ebony sapwood was 12.6 ppm, which is comparable with or somewhat higher than that in

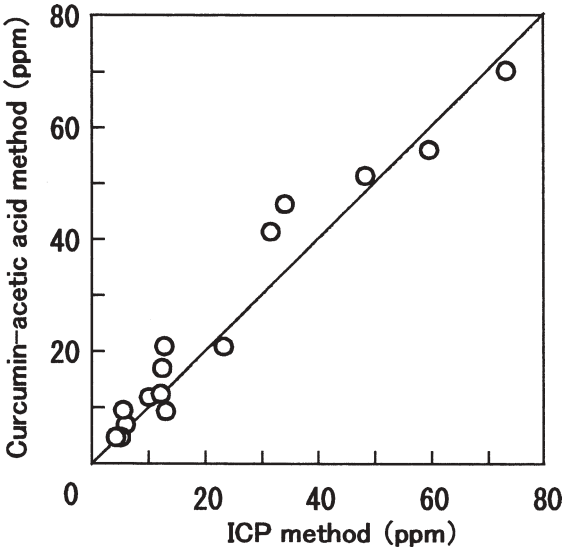

Fig. 3. Relationship between boron content determined by the ICP method and by the curcumin–acetic acid method

the whitish portion of Japanese persimmon, whereas in heartwood it was as high as that found in the blackened portion in Japanese persimmon samples 6 and 7. Consequently, the boron content of the heartwood of ebony is roughly five times higher than that of the sapwood.

The blackening of Japanese persimmon occurs under particular conditions irrelevant to heartwood formation, whereas the blackening of ebony is a common phenomenon that is associated with heartwood formation. Nevertheless, both blackened portions contained high concentrations of boron and have high resistance to fungal and termite attacks.[2,6] Consequently we arrived at a speculation that the blackening in Japanese persimmon is a result of the formation of some defensive substances in which boron may be involved. If the reaction can be carried out in vitro, it would be a novel preservation method for wood.

Comparison of ICP emission analysis with a modified curcumin–acetic acid method

Figure 3 shows the relationship between boron content determined by ICP emission spectroscopy and by the curcumin–acetic acid method. The values obtained using both methods agreed with each other, and the correlation coefficient was 0.974 for 15 samples. Because the colorimetric analysis does not need a large apparatus, the curcumin–acetic acid method is convenient and satisfactory in terms of accuracy.

Conclusions

The boron contents of Japanese persimmon and ebony were analyzed using two methods, and compared between blackened and nonblackened portions. The concentration of boron in Japanese persimmon varied among wood samples; however, for the same individuals it was always higher in the blackened portion. The boron content of heartwood of ebony was much higher than that of the sapwood. Irrespective of whether the phenomenon was common (heartwood formation in ebony) or not (blackening in Japanese persimmon), the blackened portion of *Diospyros* genus showed a high boron content, which suggests the participation of boron in the synthesis of antifungal substances. A modified curcumin–acetic acid method was substitutable for the ICP method with comparable accuracy.

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